
Metabolic Engineering of *Methylobacterium extorquens* AM1 for Conversion of Methanol to Higher Value Added Products

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Aerobic Methylotrophs

- Grow on C₁ compounds
- α, β, γ proteobacteria, *Bacillus*, *Amicrothopsis*
- Phylogenetic divisions mirror physiological classes
- Specialized metabolic pathways

Use of Methanol as a Biofeedstock

- Methanol is inexpensive, soluble in water, clean
- Methanol is produced from natural gas, but can be produced from organic wastes
- Methylophilic bacteria are amenable to genetic manipulation

Goal: to develop process strains for converting methanol to chemicals and materials biologically using methylophilic bacteria and metabolic engineering

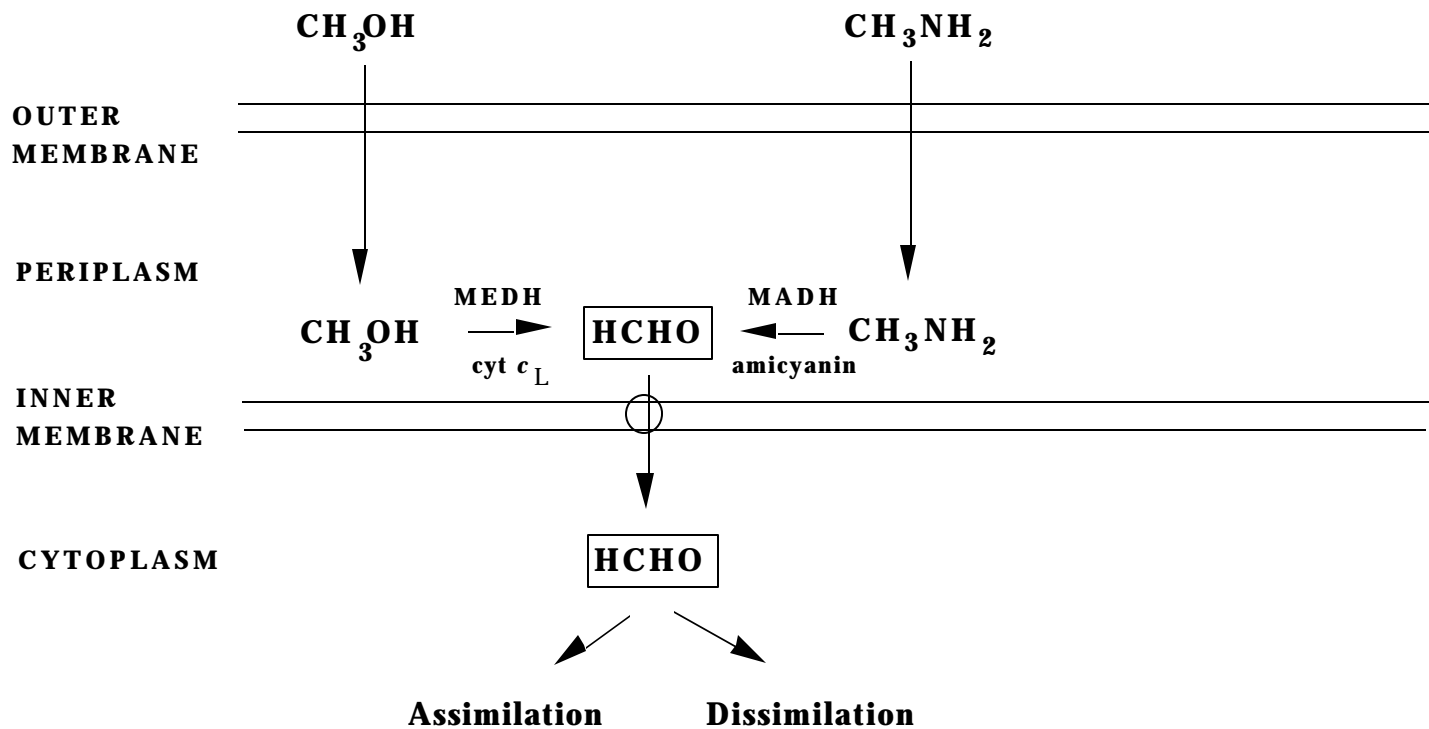
Potential Products

- Amino acids
- Industrial enzymes
- Proteins for novel materials applications
- Polyhydroxyalkanoates (PHAs)
- Polysaccharides (viscosifiers)

Methylobacterium extorquens AM1

- α -proteobacterium
- Grows on one-carbon compounds (methanol, methylamine)
- Also grows on multi-carbon compounds (succinate, pyruvate)
- Substantial toolkit for genetic analyses
 - » 110 genes identified
 - » 75 of those involved in methylotrophy

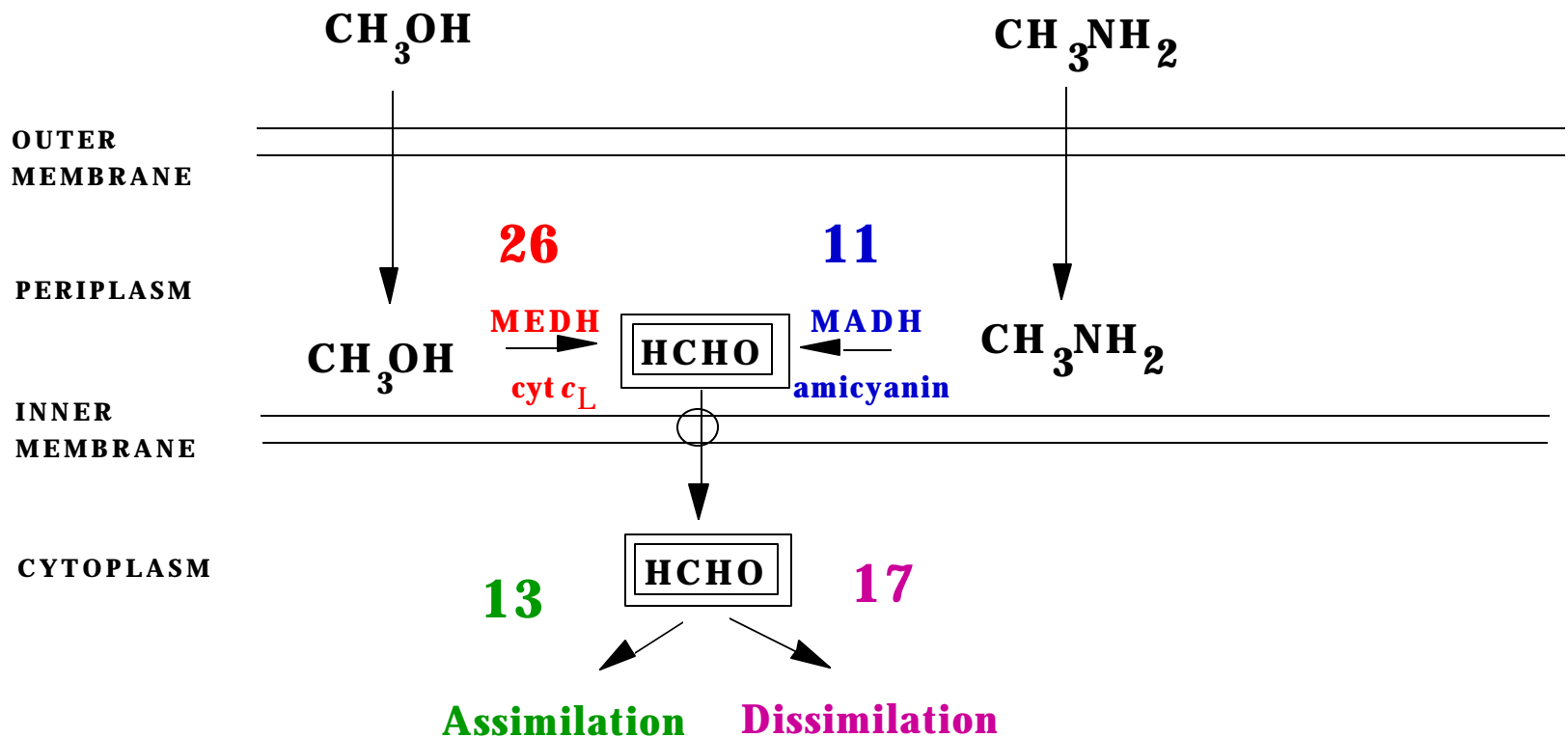
Methylotrophic Metabolism



MEDH = methanol dehydrogenase

MADH = methylamine dehydrogenase

Genes Involved in Methylotrophic Metabolism



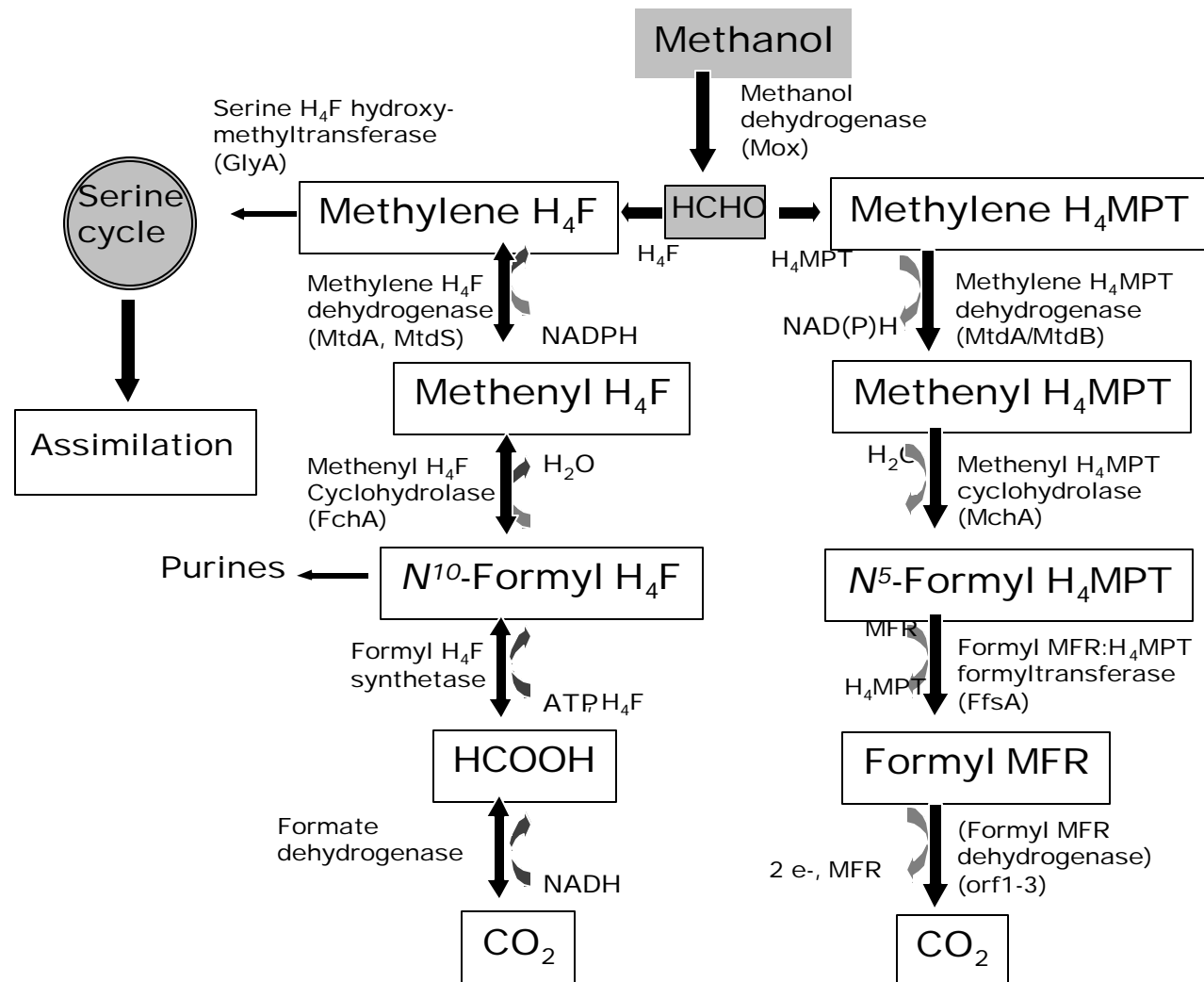
UNKNOWN: 8

TOTAL: 75

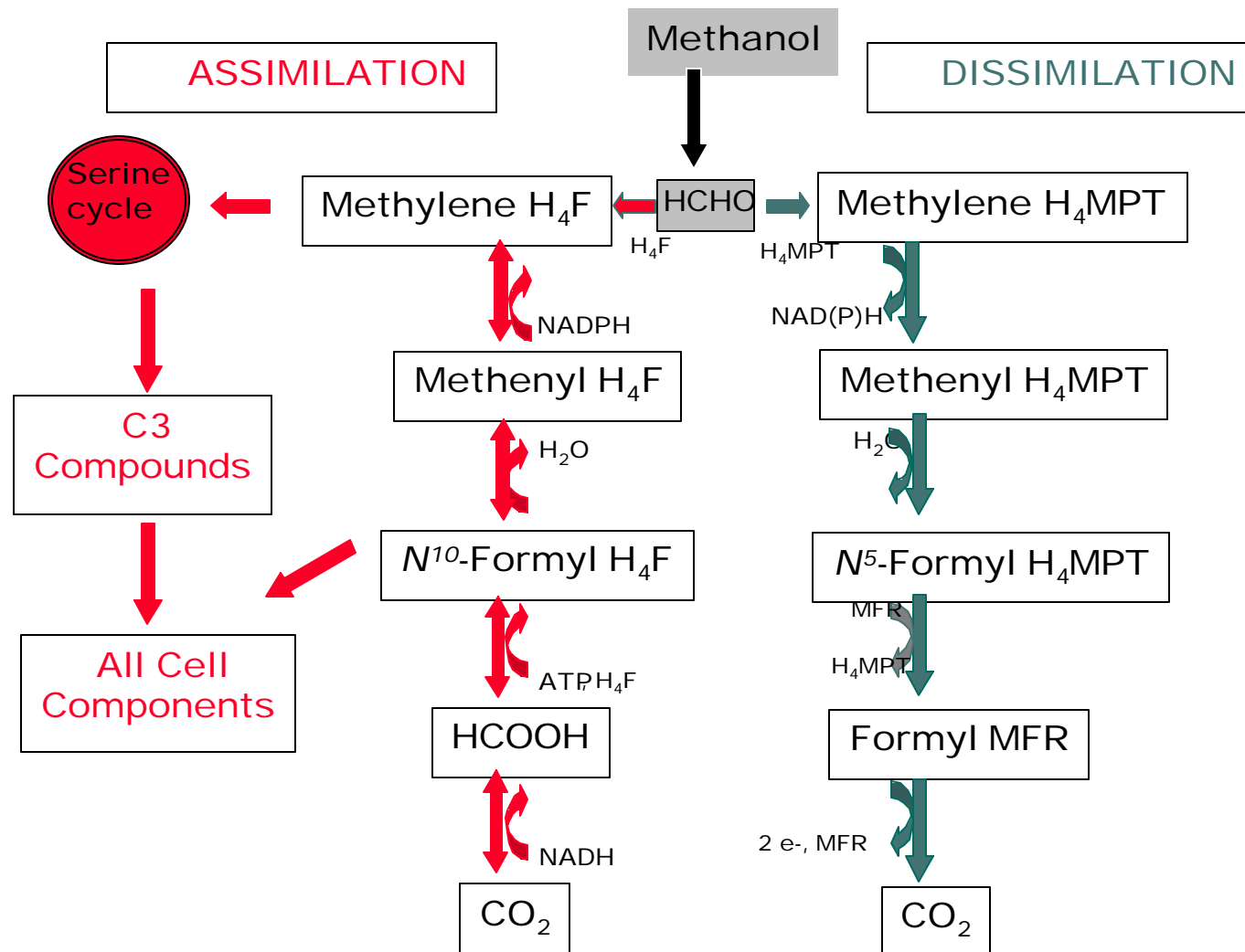
Genome Project in *M. extorquens* AM1

- Funded by General Medical Sciences (NIH)
- Joint with UW Genome Center (Maynard Olson)
- 6 Mbp genome, 68% GC
- 130 kb available sequence
- Rationale
 - » Gene discovery
 - » Develop tools for genome-wide metabolic analysis and metabolic engineering

Two Folate-linked Pathways in Methylootrophs



Flow of Carbon to Intermediates and Polymers



Formaldehyde-handling Issues

- Methylotrophs that grow well on high methanol have multiple formaldehyde handling pathways
- The pathways are not redundant: mutants in each pathway become methanol-sensitive
- The central issue for metabolic engineering of methylotrophy is handling formaldehyde flux

Why a *Methylobacterium* and Not a Different Methylophil?

- Best at handling formaldehyde
- Genetics is most facile
- Underlying metabolism much more versatile

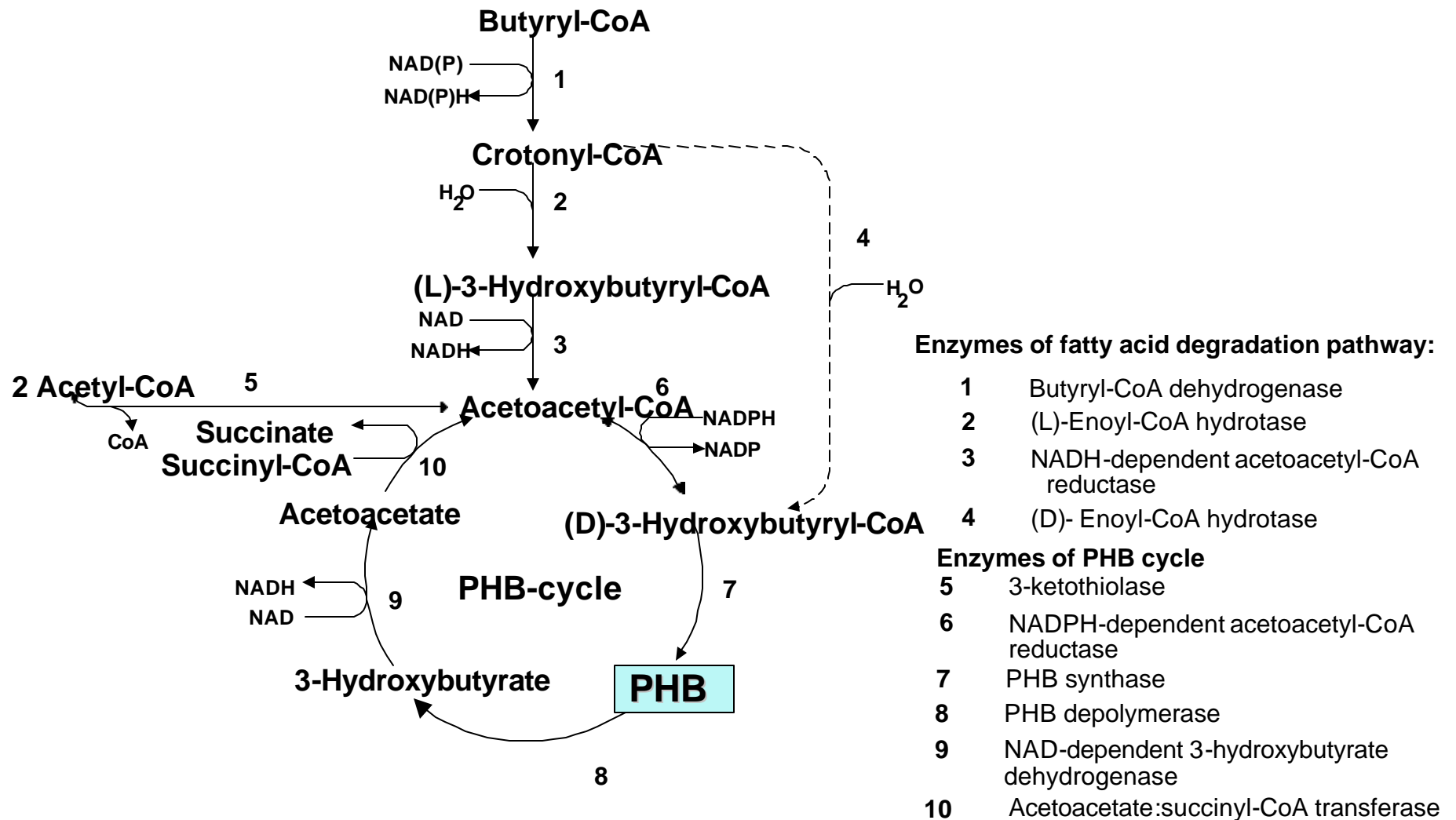
PHB Synthesis in *M. extorquens* AM1 (% of dry wt)

- Methanol-grown cells (exp.): 40%
- Succinate-grown cells (exp.): 15-20%
- Methanol-grown cells (N-lim): 80%

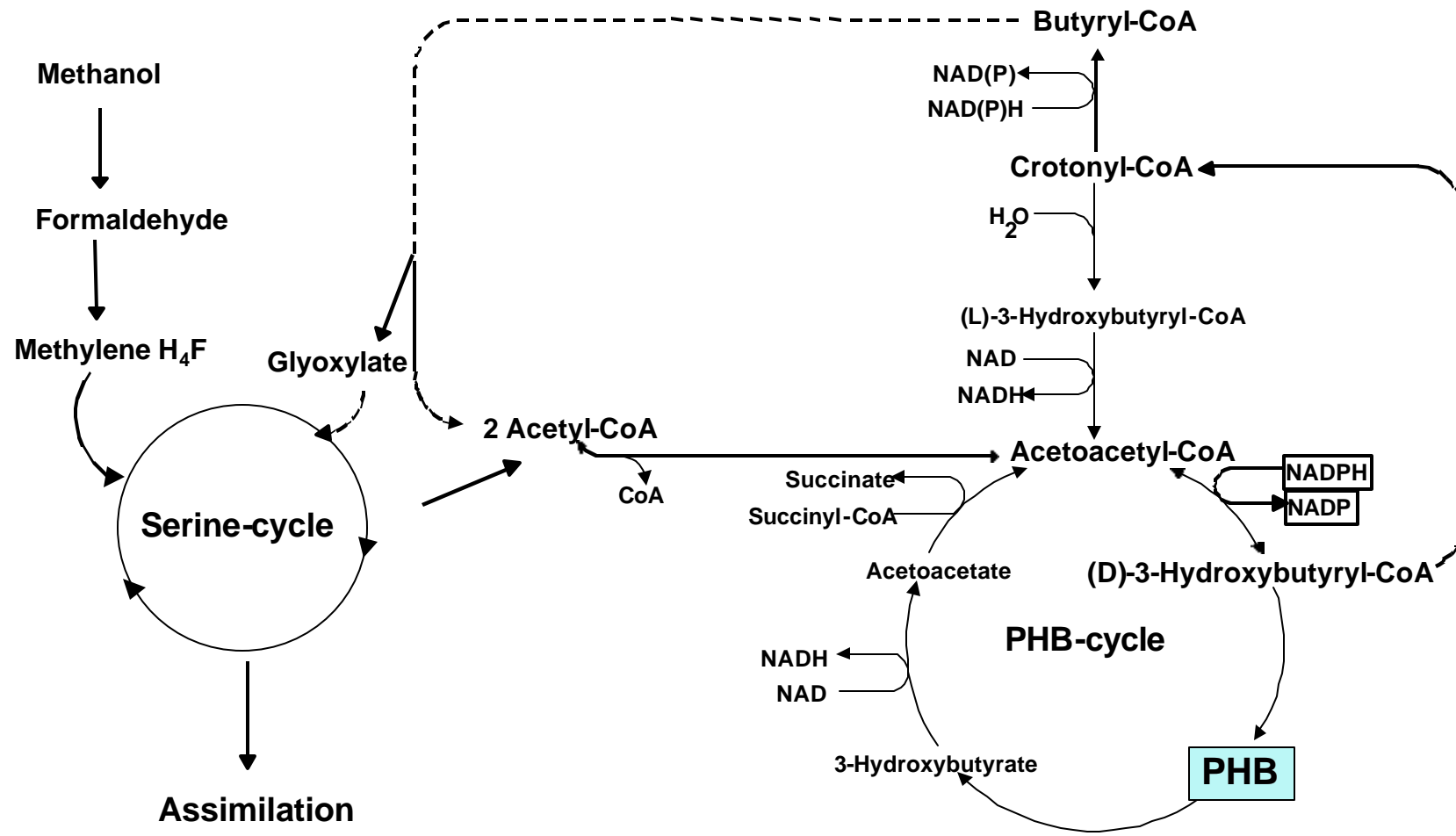
Approach

- Systems-level understanding of methylotrophy (mutants and wild-type)
 - » Metabolic reconstruction
 - » Expression arrays
 - » Proteomics
- Metabolic modeling
 - » Formaldehyde flux through multiple pathways
- “Classical” physiological studies (multi-variant functional analysis)

The PHB Cycle in *M. extorquens* AM1



The PHB Cycle in *M. extorquens* AM1: Connection to Methylotrophy



Summary

- Methylotrophs have unusual metabolic capabilities of interest for biocatalytic applications
- Genetic manipulation is possible, especially in the *Methylobacterium* strains
- Genome-level analysis opens the opportunity to develop process strains for converting methanol to higher value added products